

Fig. 1. Schematic diagram of methoxy mycolic acid synthase mmaA 4-mmaA 1 gene cluster of mycobacteria and location of forward A, and reverse D primers.

CTACTTCGCCAGCGTGAACGGTTGACGTCGATGTAGCCGACCCGAAACAGCTGGCGCAGCC
GGTCAGGTATTCATGTACCGCTCGTAGACCTCTCGGACTGGATCGCATGGCCTCGCTTGTGTT
CTGCAGCGCCTCGGCCACAGGTCGAGGGCCTGGCGTAATGCGGCTGCAGCGACTGGCGGAGTCA
GCGTGAACCGTCTCGCCACTGTTCTCAACCATTCAATCGTCGGAGGTTGGCCCCCGGGAAAG
ATTTCGGTCGCGATGAACCTGAGAAAGCGGGCCAGCCACAACGTGAGCGGCAAGCCGTGGTCGACCA
TCTGCTGCCTGGTCAGGCCGGTGATCGTGTGCAGCAGCAACACGCCATGGCGGAGGATTGTGG
GCCCGGGCGAAGAACGTCGGCGTACGATCGTGGCGAAGTGCTGAACCGCCGATCGACACGATGC
GGTCGACGGGCTGTTGAACTGCTCCATCCGCCAGCAACACTCGCCTGTCGCGCGGGGTGTCCATC
TCGTCGAACGACTTCTGCACATGGCGGCCTGGTCTTCGACAATGTCAGGCCGACGACGTTGACGTC
ATACTGCGCGATCGCGCCGCATGGTGGCGCCCCAGCCGAAACCGATATCGAGCAGCGTCATGCCGG
GCTGCAGACCTAGCTGCCAGCGCCAGGTCGATCTGGCGATCTGGCCTCTCCAGCGTCATGTCCT
CGCGTTGAAATGCGCGCAGCTGTAGGTCTGGTCGGATCCAGGAACAGCCGGAAGAACGTCGCGA
CAGGTCGTAGTGTGCCTGCACGTCTCGAAGTGCAGCGTTAGGTCGTTGACCA*Tgaggtgtaa*tgcttccggaccct
aggtggccttcggtgctgcacggAACGcaccgatgtcccccccccgcattcgaggcatgtatccgata
caggccgcactaaaccgcgatcgaaatttgc
ccaggcaggaaacggatatgagcggacgagCTACTTGGTCATGGTAACGGCGACGTTGATTAGGCCT
CGCGTCCCGCATCCGGCAGATAGTCATGAAGTTGTTGAGACCTCTCGGACTGTACGGCGATGG
CGCGTTGCGGGCAGCCTGTAGGTTGGCGGCCATGCATCGAGAGTCCGTGCGTAGT*GCTGCTGCAGCA*
GCTGGACATGCTCGATGGTAAGCCCAGGCCTGCGATTGTCGACAATGTCGGCTCCGATGGCAGC
TCGCCGCCGGAAAGATCGACTCCGCAGGAATTGAGGAATCGAAGGTCGCTCATCGTCAGCGCAAT
GCCCTGTTGTCAGGCCACCTGCGGTCGTAGGTGAACAGGCTGTGCAGTAGCATCCGCCGT
GCAGGATGTCGTAGGAGCGTTCGAAGAACGTCAGATACCGCTCCTTTGAACCGCTCGAATGCC
AAAGCTGACGATCCGGTCGACGTTCTCTCAAACCTTCCAGCCCTGCAGCCGGCCTCGCGCGCG
TGCCTCCGATTGCGGCCAGGGGTCTTGCTGCGTTCATAGTGAATTCCGGCTGAGCGTGAGGCC
ACATTGACGTCGTACTTCTCCACGGCCGAACGAGCGCCCCACCGCAACCCACGTCGAGTAG
CGTCATCCCCGGTTCGAGGTTAGCTTCCAACGCCAGATCCACCTGGCCAGTTGCGCCT
CGTCATATCGTCACGCTCGAAATAGGCGCAGGTGTAGACCCAGGTGGATCGAGGAACACGCGAAG
AAGTCATCCGAAATGCGTAAGCCACTGTACTCTCGTAATATGGTCTCAGCTGGCCAT

Fig. 2. Sequence of mmaA2 and mmaA1 gene with an intergenic region of 166 base pair (shown in lower case. Location of forward A, sequence ID 1 and reverse primer D, sequence ID 2. Both primer sequences are underlined and italicized.

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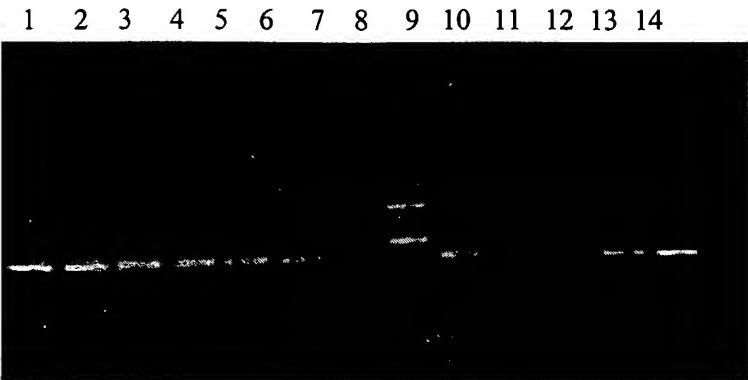


Fig. 3. PCR amplification of different mycobacterial genomic DNAs with primers A and D (lanes 1- 15); 1. *M.avium* 2. *M.bovis* 3. *M.chelonae* 4. *M.fortuitum* 5. *M.intracellulare* 6. *M.kansassi* 7. *M.phlei* 8. 100 bp DNA ladder 9. *M.marinum* 10. *M.scrofulaceum* 11.*M.smegmatis* 12. *M.szulgai*, 13. *M.tuberculosis* and 14. negative control. AD indicates 363 bp-amplified product.

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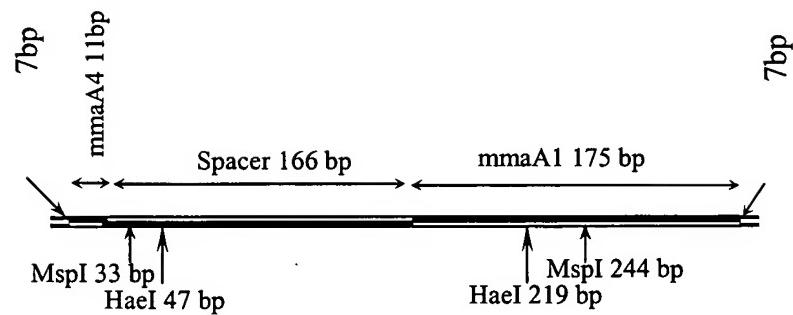


Fig. 4. Line diagram showing restriction endonuclease map of HaeI and MspI within AD.

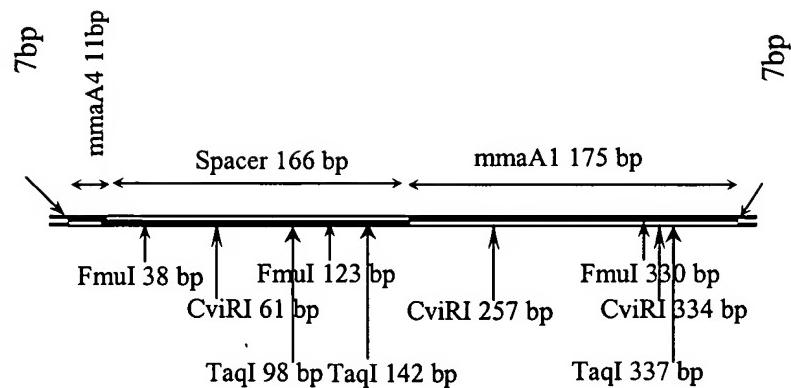


Fig. 5. Line diagram showing restriction endonuclease map of FmuI, CviRI and TaqI within AD.

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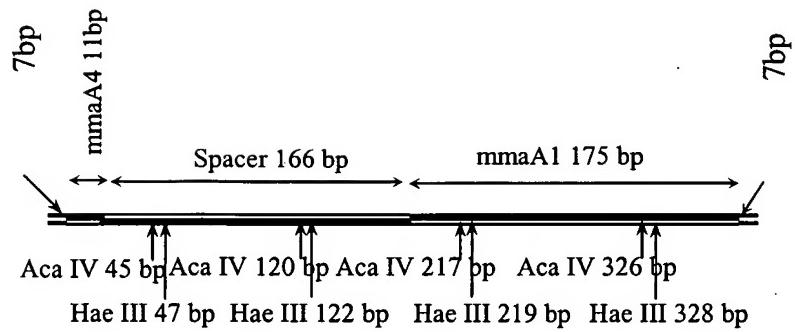


Fig. 6. Restriction map of AD showing distribution of the sites of restriction endonucleases AcaIV and HaeIII.

ARTICLE I

ARTICLE II

ARTICLE III

ARTICLE IV		Steps	Temp	Time	One
		Denaturation	95°C	3 min	
14cycles		Denaturation	94°C	45 sec	
		Annealing	70°C (-0.8°C /cycle)	45 sec	
		Extension	70°C	1min	
25 cycles		Denaturation	94°C	45 sec	
		Annealing	58°C	45 sec	
		Extension	72°C	1min	

Diagram illustrating the PCR reaction steps:

- 14 cycles: A loop arrow labeled "Touch Down Cycles" indicates the first 14 cycles.
- 25 cycles: A loop arrow labeled "Normal PCR Cycles" indicates the remaining 25 cycles.

Fig. 7. Line diagram showing different steps of PCR reaction